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The prognostic significance of vascular endothelial growth factor levels in sera of non-small cell lung cancer patients

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Summary *Background*: Angiogenesis, the formation of new blood vessels from the existing vascular bed, is essential step for the growth and invasion of the primary tumor. Vascular endothelial growth factor (VEGF) is known to play crucial role in tumor angiogenesis. Increased serum VEGF levels may be associated with poor prognosis in patients with non-small cell lung cancer (NSCLC).

Methodology: In the present study, we measured plasma VEGF levels in 20 normal subjects and 75 patients with untreated NSCLC; 23 operable (stages I, II, IIIA) and 52 inoperable (stages IIIB, IV) (Histology: squamous cell carcinoma, 40; adenocarcinoma, 27; undetermined, 8). VEGF was measured by enzyme-linked immunosorbent assay.

Results : The median VEGF level in patient group was 119 pg/ml (29–1235), which was significantly higher than the control group ($P=0.044$). Median survival of patients was 210 days (30–220). The patients were divided into high VEGF (>119 pg/ml) and low VEGF (<119 pg/ml) groups using the median value as a cut-off. It was investigated if there were significant associations between serum VEGF level and clinico-pathological parameters like age, sex, histopathological diagnosis and TNM staging. Also high VEGF and low VEGF patient groups were compared according to the median survival.

Conclusions: Serum VEGF level is significantly associated with the clinical staging of the patients (operable and inoperable) ($P=0.031$) and it also correlates with the prognosis of the patients ($P=0.0006$).

Introduction

It is well established that angiogenesis plays a pivotal role in the growth of the neoplasm. It also facilitates the progress and metastasis of tumor, so the quantity of intratumoral neoangiogenesis is related to prognosis. It was also shown that there is strong correlation between density of microvessels in the primary tumor and its metastatic capability.[1–7]

Neoangiogenesis promotes the tumor progression by delivering nutrients and oxygen which is essential for growth, facilitating the penetration

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of tumor cells through the vessel wall and their transport to distant organs and secreting growth factors and cytokines from endothelial cells that stimulate the tumor cells.[2,4,8,9]

The process of neovascularization is a complex series of events including endothelial cell migration, proliferation, differentiation in addition to extracellular matrix proteolysis.[6,7] This process is in equilibrium with stimulatory and inhibitory factors.[8]

Tumor cells secrete a variety of stimulating factors for microangiogenesis including fibroblast growth factor, transforming growth factor, platelet derived endothelial growth factor and hepatocyte growth factor. Among these angiogenic factors, Vascular endothelial growth (VEGF) is an endothelial cell specific potent mitogen.[6,7,9] It is a soluble dimeric 34–46 kD protein involved in endothelial cell proliferation and migration, increased vascular permeability and stromal degradation through the activation of proteolytic enzymes.[4,5,8,9] VEGF expression has been demonstrated in some malignancies including lung cancer and there are studies that increased VEGF expression is correlated with poor prognosis.[8,9]

Lung cancer is the leading cause of death among malignancies and non-small cell lung cancer (NSCLC) accounts for more than 75% of pulmonary malignancies.[8] NSCLC shows heterogenity for its histopathological classification and behaviour. More than half of the patients relapse after complete resection, indicating that NSCLC is a disseminated disease from the beginning in the majority of patients. NSCLC prognosis is strongly influenced by clinico-pathological parameters such as performance status, tumor status, nodal metastatic involvement and distant metastasis and yet the TNM classification is the best predictor of outcome for the patients.[8,10] However, it is known that the prognosis may vary considerably within each stage of disease. Therefore, new prognostic factors must be found to assess individual risk profiles.

It is thought that angiogenesis plays an important role in NSCLC where distant organ metastasis and regional nodal involvement is frequent. VEGF expression has been reported in 50–95% of NSCLC patients. Various investigators have shown the correlation between VEGF expression and poor prognosis, nodal metastasis and vessel density.[8,9,11,12]

The exact origin of VEGF in sera of patients is still unclear. It is thought that the malignant cells themselves and inflammatory cells infiltrating tumor are the main sources of VEGF as well as peripheral blood cells like thrombocytes and neutrophils.

There are few studies focussed on the levels of VEGF in sera of lung cancer patients and its effect on survival. In the present study, we aimed to analyse the correlation between serum VEGF levels in NSCLC patients and different clinico-pathological parameters and prognosis.

Materials and methods

This study consisted of 20 healthy controls with no known systemic disease, all matching with age and sex with the patient group and 75 NSCLC patients. All subjects were enrolled in our department between January 2001 and January 2002.

All patients were evaluated with a complete physical examination and Karnofsky performance scoring. Each patient underwent the following staging procedures: chest radiography, chest computed tomography (CT) scan, fibreoptic bronchoscopy, radionuclide bone scan and abdominal ultrasound. Cranial CT scanning was performed only if the patient had neurological symptoms. Patient samples were collected right after the diagnosis and none of them have received any therapy prior to sample collection.

After collection of the venous blood samples from patients and control subjects, the samples were centrifugated at 3000 rpm for 10 min and then stored at -70°C. The serum samples were assayed for the level of VEGF by a sandwich enzyme-linked immunosorbent assay (ELISA), according to the recommendation of the manufacturer with a human VEGF immunoassay kit (Cytimmun Sciences Inc, Maryland, USA). Each serum sample was assayed twice and minimum detectable VEGF concentration was <18.6 pg/ml, variation range coefficient was between 8.9% and 11.1%.

Statistical analysis

The Mann-Whitney U and χ^2-test were used to compare different groups for continuous variables and for cathegorical variables respectively.

Dates of diagnosis and death of all patients were recorded. Overall survival was defined as the time from the day of diagnosis to death; data on survivors were accepted as the day the study was ended. Overall survival was calculated using Kaplan-Meier Method. Survival curves were compared with a log-rank test. Kruskal-Wallis test was used to compare serum VEGF levels and tumor stage.

All statistical analyses were performed using the SPSS version 8.0 statistical program (SPSS Inc, Chicago, Illinois, USA).

Results

Patient characteristics

In the NSCLC group median age was 62 (38–84) and group consisted of 66 male (88%) and 9 female (12%) patients.

Patient staging was made according to American Joint Committee on Cancer. According to the staging, at the time of diagnosis 23 patients (30.7%) were operable (Stages I, II, IIIA) and 52 were inoperable (stages IIIB, IV). Majority of patients (53.3%) had squamous cell carcinoma. Patient characteristics were summarized in Table 1.

Serum VEGF levels and patient characteristics

In the patient group, median VEGF level was 119 pg/ml (29–1235 pg/ml). This was significantly higher than the controls ($P = 0.044$).

The patients were divided into high VEGF (>119 pg/ml) and low VEGF (<119 pg/ml) groups using the median value as a cut-off.

There were no significant associations between serum VEGF levels and various clinico-pathological parameters like age, sex and histopathology ($P>0.05$).

Table 1 Patient characteristics.

	Low VEGF group ($\leqslant$119) (38)	High VEGF group ($>$119) (37)	$P =$
Age			
$\leqslant$62	22	18	>0.05
>62	16	19	
Sex			
Male	34	32	>0.05
Female	4	5	
Histopathology			
Squamous cell	21	19	>0.05
Adeno Ca	11	14	
Undetermined	5	3	
Large cell Ca	1	(−)	
Bronchooalveoler Ca	(−)	1	
Stage			
IA	2	3	>0.05
IB	5	1	
IIA	(−)	(−)	
IIB	2	1	
IIIA	7	3	
IIIB	10	13	
IV	12	17	
Operable:	16	7	$=0.031^*$
Inoperable:	22	30	
Performance status			
%100−80	26	17	>0.05
%70−50	11	19	
$\leqslant$%40	1	1	
Median Survival	285 days (30–660 days)	150 days (30–510 days)	$=0.02^*$

$^*P<0.05$.

According to the definitions in TNM staging system, each T, N, M values were compared with the serum VEGF levels. When the patients were grouped as T1-2 and T3-4 according to the primary tumor size, there was significant difference between these two groups for serum VEGF levels ($P = 0.042$). However, no significant association were shown between serum VEGF levels and presence of regional lymph node metastasis or distant metastasis.

When patients were grouped as operable and inoperable, there were statistically significant difference between two groups for serum VEGF levels ($P = 0.031$) (Fig. 1). In the operable group median serum VEGF level was 73 pg/ml (29.1–614 pg/ml), while in the inoperable group median level was 131 pg/ml (29–1235 pg/ml). In the patient group, 69.3% of patients had serum VEGF levels higher than the median level of the control group which is 68.6 pg/ml. There were no significant association between serum VEGF levels and histopathological type of tumor ($P > 0.05$).

Serum VEGF levels and survival

Eight patients in the study group were lost to follow up. Remaining 67 patients had median survival of 210 days (30–220 + days) (+, shows that patient still lives). When the study was finished, four patients were still alive.

In the operable group (22 patients), median survival was 225 days (30–540 +) while in the inoperable group 210 days (60–660 days).

We have found moderate negative correlation between serum VEGF levels and survival (Spearman's correlation coefficient $(r) = -0.46$, $P < 0.001$) (Fig. 2).

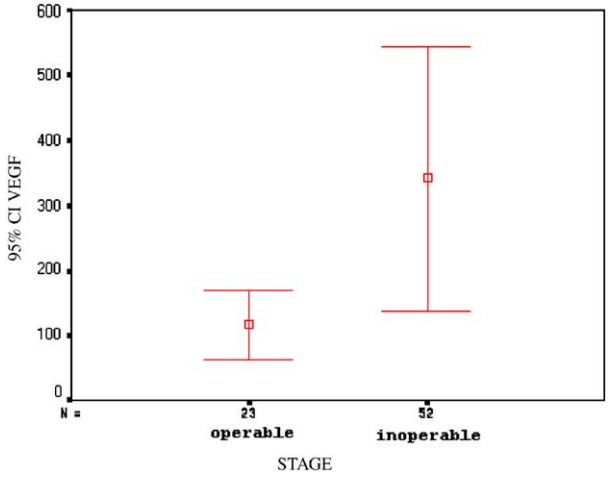

Figure 1 Distribution of median serum VEGF levels between operable and inoperable groups.

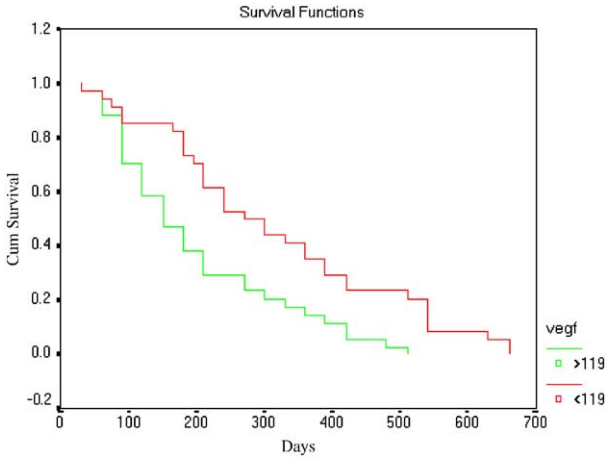

Figure 2 Kaplan–Meier survival curve according to median VEGF levels of 67 patients.

According to Cox regression analysis, serum VEGF level was found to be a risk factor on the survival independent of the *T* factor (relative risk = 1.78, 95% CI = 1.027–3.084, $P = 0.04$).

Discussion

NSCLC is a lung cancer subgroup of particular interest for its histopathological classification and behaviour.[7] More than half of the patients relapse after complete resection, and this high recurrence rate is attributable to microscopic metastases present at the time of initial diagnosis.[9] This high metastatic potential of NSCLC may be due to angiogenesis of tumor in which VEGF plays an important role.

In our patient group, we found higher serum VEGF levels compared to the healthy controls and these high levels were shown to be statistically significant. Similar results were obtained in some malignancies including lung cancer previously.[8,9,13]

In our study, like other similar studies, median and mean VEGF levels had high standart deviations. We think that the heterogenity of the NSCLC itself and the difficulties in using serum VEGF as a biological marker might be the underlying cause.

While there is an association between serum VEGF and tumor staging in some studies,[8,14] there was no such association in others.[9,13] In our study group, the distribution of patients according to tumor staging was not homogenous. Thus, patients were grouped according to the preferred therapy as operable and inoperable. Although we could not

show a significant association between tumor stages (each N, M) according to TNM staging system, and serum VEGF levels; there was significant difference between operable and inoperable group by means of serum VEGF levels ($P = 0.031$).

There are several hypothesises for the origin of VEGF in serum. In our study, we showed that serum VEGF levels for T1-2 patients (29 patients, 38.73%) were higher than the T3-4 (46 patients, 61.3%) ones. This finding was consistent with the previous studies indicating a positive correlation between serum VEGF levels and the primary tumor size. Also this finding supports the hypothesis that tumoral cells themselves are the main source of VEGF and primary tumor size is important in detecting the serum VEGF levels.[12]

There was no association between serum VEGF levels and histological types of NSCLC in our study, as none of the previous studies have shown any.[8,9,13]

Limited data is available on the prognostic significance of serum or plasma VEGF levels in NSCLC. In various solid tumors, immunohistochemistry or ELISA is commonly used for prognostic significance of VEGF. Especially in some hematological malignancies, it has been documented that VEGF overexpression is together with early relapse and poor prognosis.[10,12] But in majority of studies concerning lung cancer, no prognostic significance of serum VEGF on survival has been shown. Decaussin et al.[15] have measured the microvessel density (MVD) and VEGF expression on the tumoral tissue specimen of 69 Stage I-II NSCLC patients and they were not able to demonstrate the effect of MVD or VEGF expression on prognosis. However, in our study, it is shown that survival of patients with serum VEGF lower than 119 pg/ml is longer than the patients with high serum VEGF levels. We think that this result is due to our larger patient group.

In conclusion, we have demonstrated that serum VEGF levels are higher as the tumor stage progresses and tumor size increases, so naturally we had lower serum VEGF levels in the operable patient group. We think that, in operable NSCLC patients who has high serum VEGF levels, neutrolizing antibodies targetted against VEGF together with adjuvant chemotherapy prior to surgery may increase survival and better prognostic outcomes might be achieved.

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